

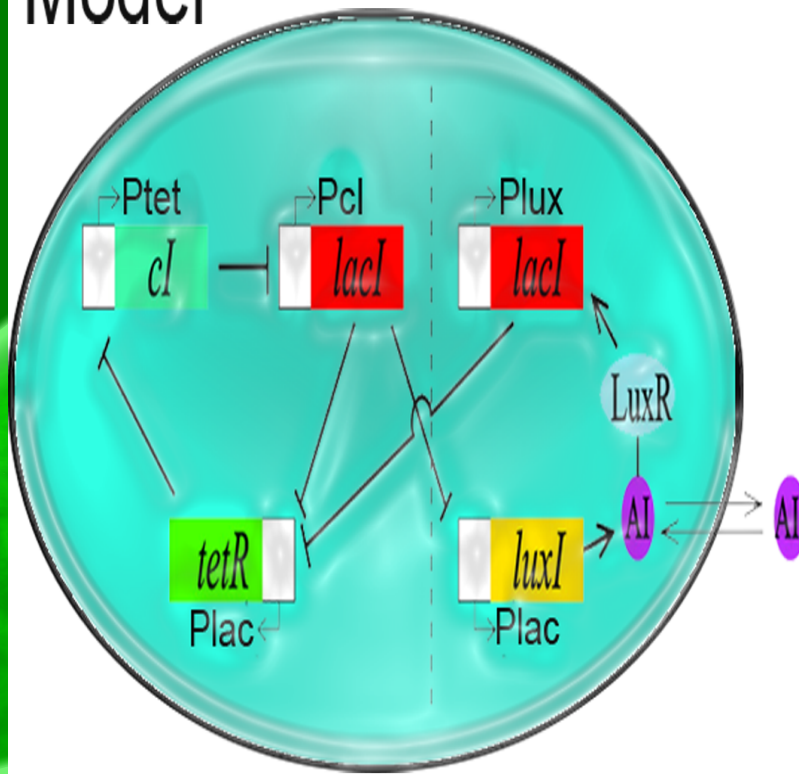
Oscillator with quorum sensing: “L’ e. coli de danse”

IGEM Team 2

*Presented by: Jamie Schafer,
Horia Vulpe*

Inspiration: Garcia-Ojalvo et al. PNAS101(30):10955(2004)

Model



- A standard oscillator is coupled to the Lux quorum sensor
- This results in synchronization of oscillations in coupled cells
- Formation of LuxR is assumed to be constitutive

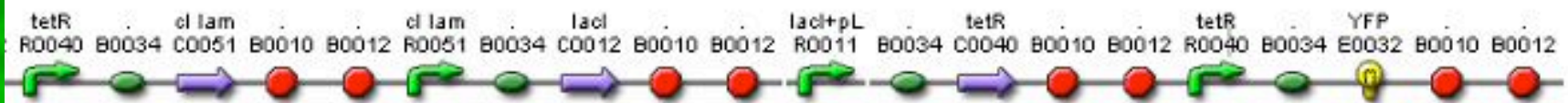
Testing this model with BioBricks



I15004, available with Kan resistance

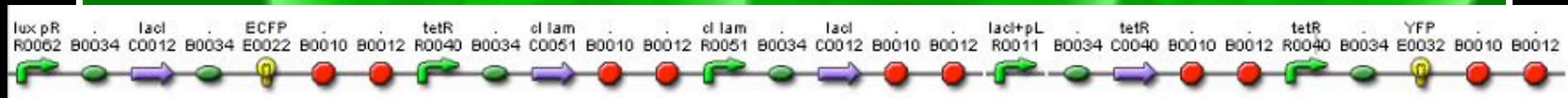


J40001 made from S0100, E0422 and R0062. AmpR



Entire Elowitz repressilator, available as I5610 with YFPaav

Ligate repressilator to J40001 to yield full system on 2 plasmids with Amp/Kan

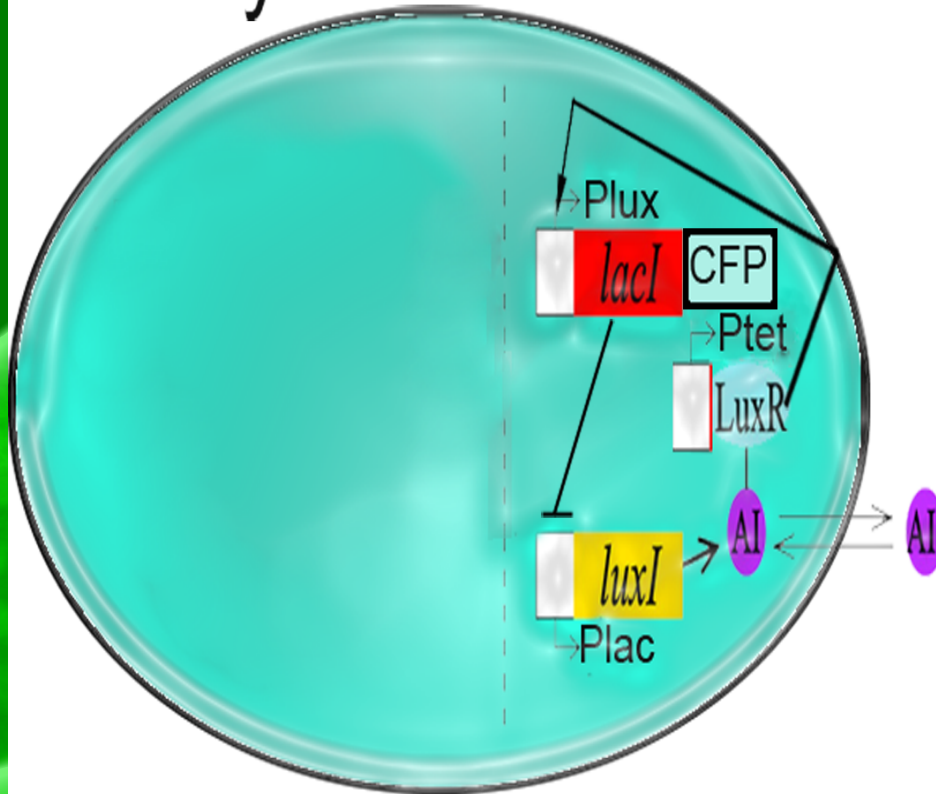


J40007 ligation had issues:

- Restriction digest of Repressilator fragment did not yield expected fragments on gel
- Other incarnations of the repressilator gave the right fragments, but either had no fluorescent reporter gene or the reporter gene was non-destabilized (I5611, I5612)

Test System 1: no repressilator

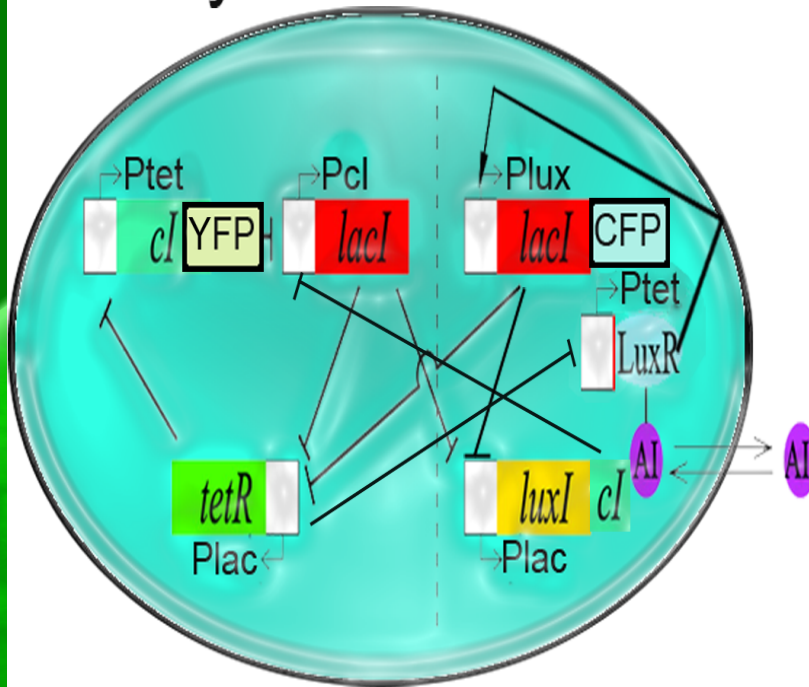
Test system #1



- Two promoter oscillator- coupled
- Output is destabilized CFP
- Quorum sensing possible

Full system: more complicated than model!

Test system #2



- Two additional couplings not present in model
- *Plac* makes *cl* as well as *LuxI*
- *LuxR* is not constitutive, but is under *pTet*, as is *cl*-YFP

Simulations: approach

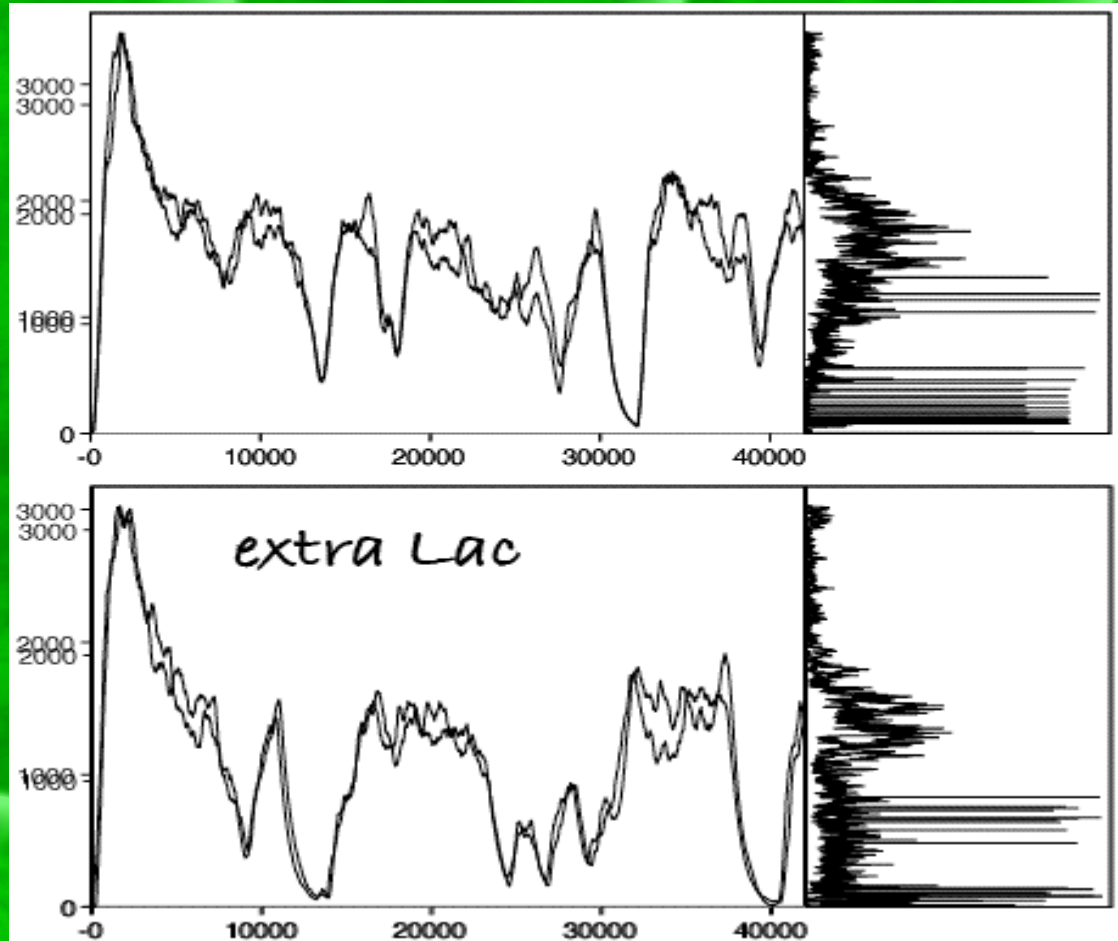
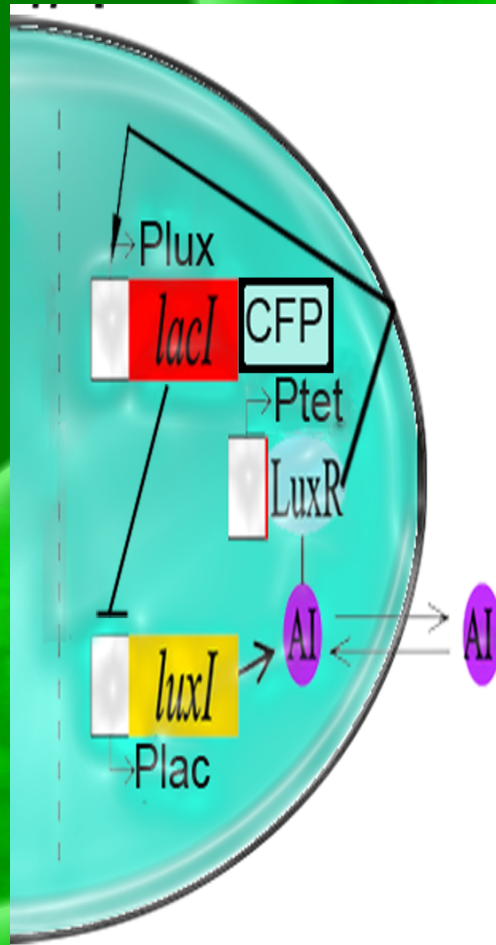
Methodology

- BioNets used to model reaction rates
- All continuum variables except promoters
- Rates and assumptions taken from Elowitz 2000

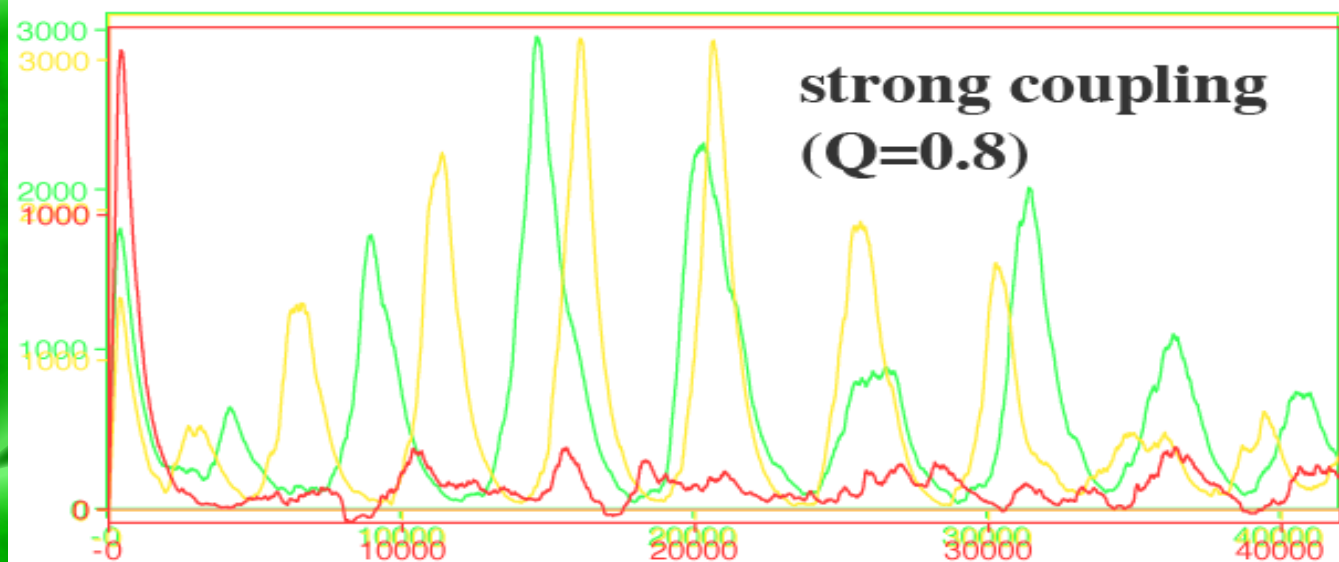
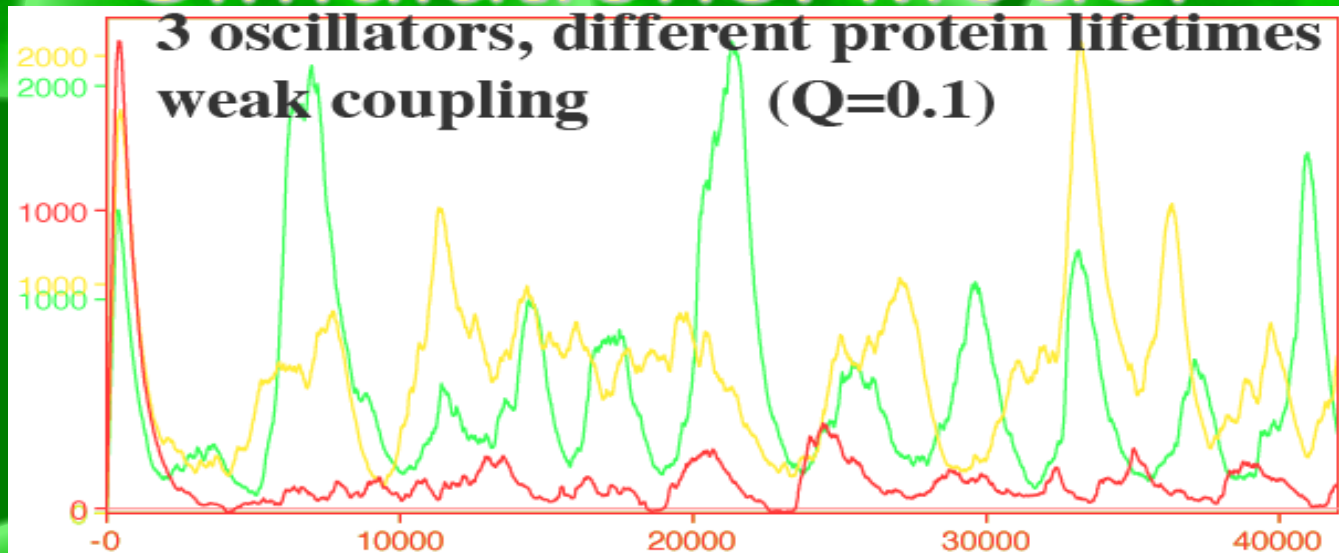
Issues/observations

- Models very sensitive to Plux rates
- Unsure whether protein or mRNA lifetimes should vary
- Synchronization not seen in model in most cases

Simulations: Test System 1

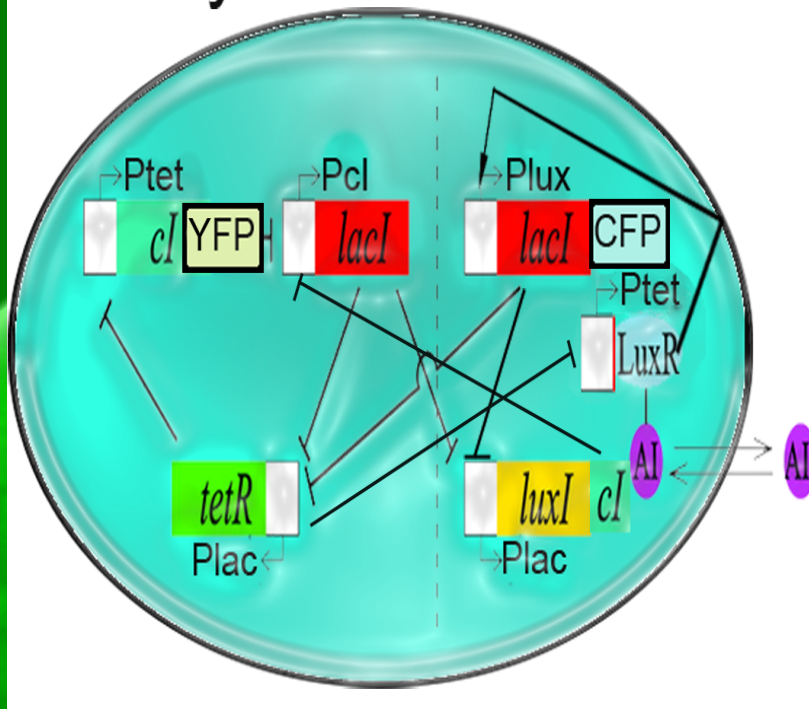


Simulations: Model



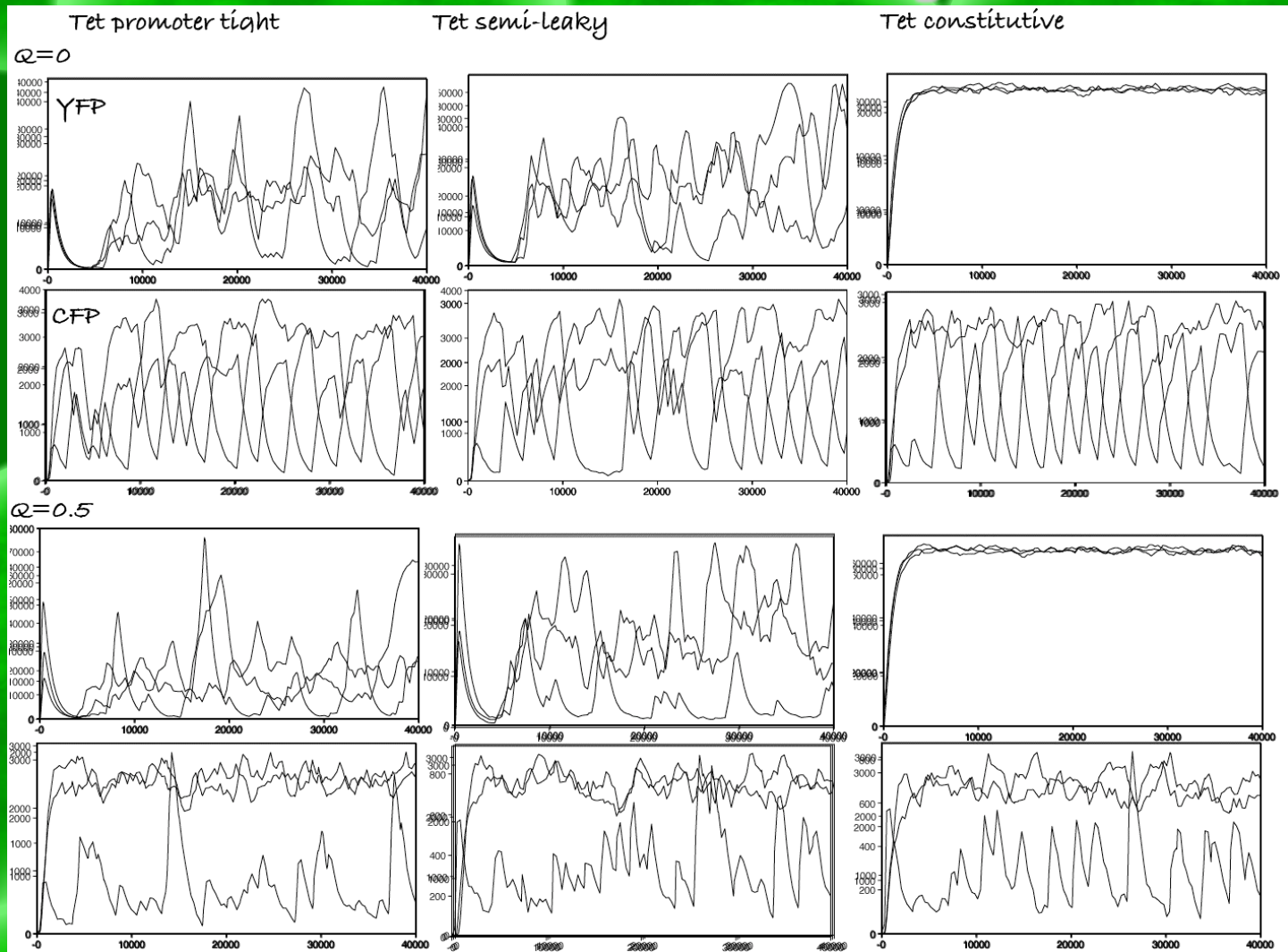
Test System 2

Test system #2

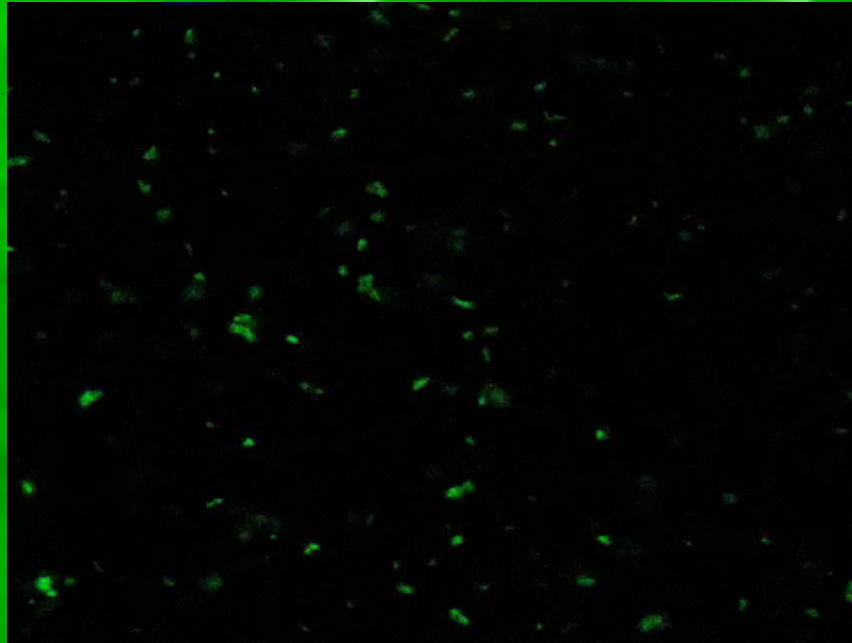


- TWO fluorescent proteins to be monitored: CFP and YFP

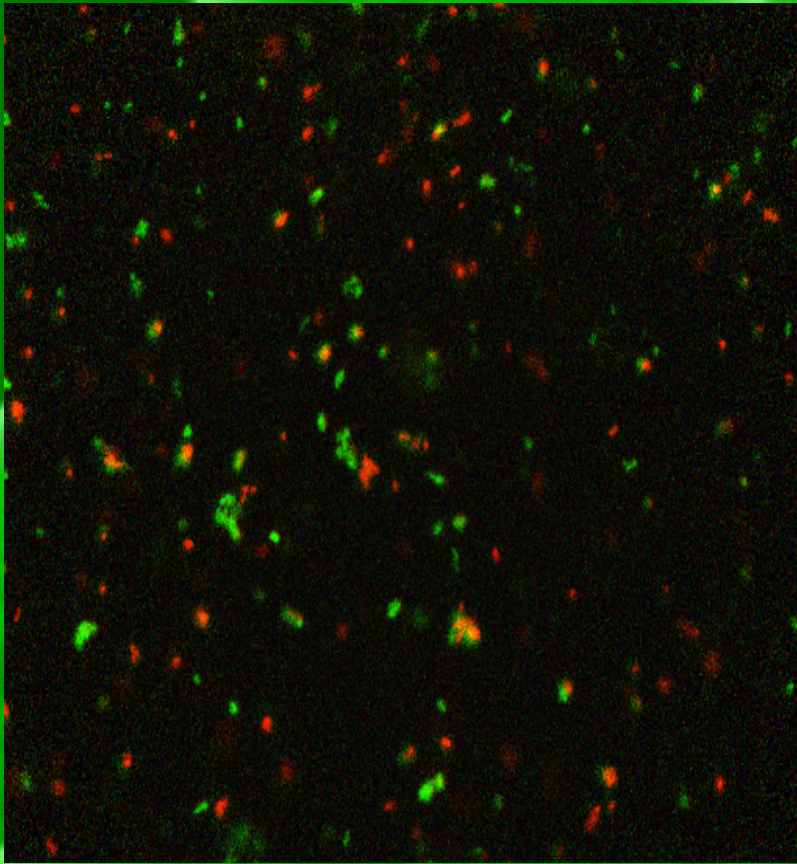
Simulations: Test System 2



Results: Test System 1



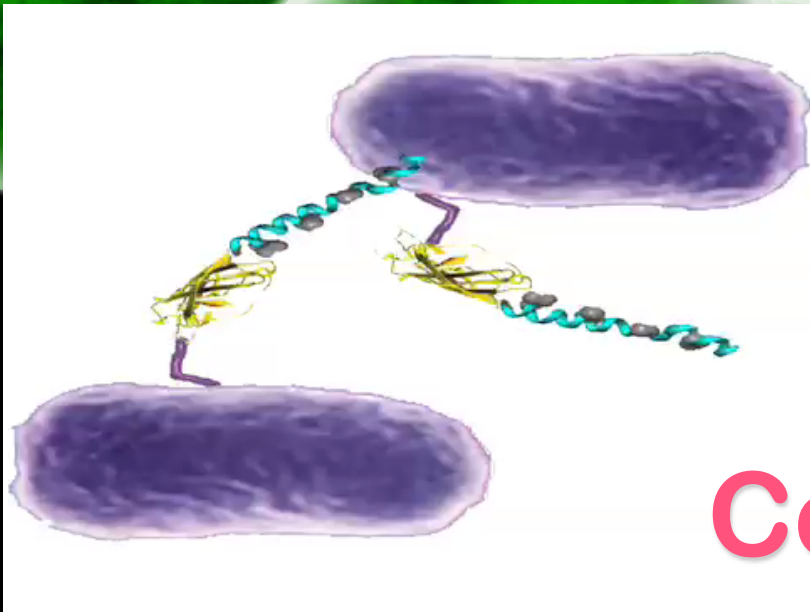
Interpretation



- Cells in green begin in synch
- Cells in red begin out of synch and synchronize

Conclusions/future work

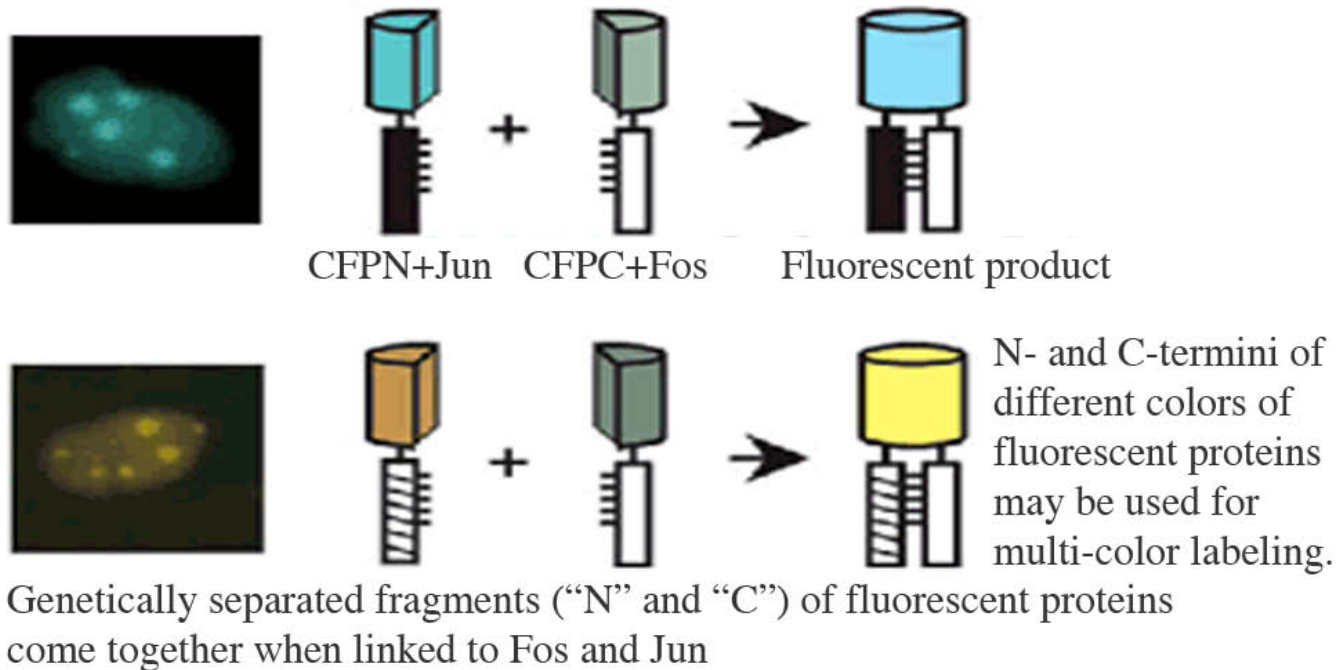
- Synchronization is seen
- Expected with more complicated system
- Will need to image CFP/YFP simultaneously
- Use repressilator alone as a control



IGEM Team 1: Extracellular Fluorescence Complementation

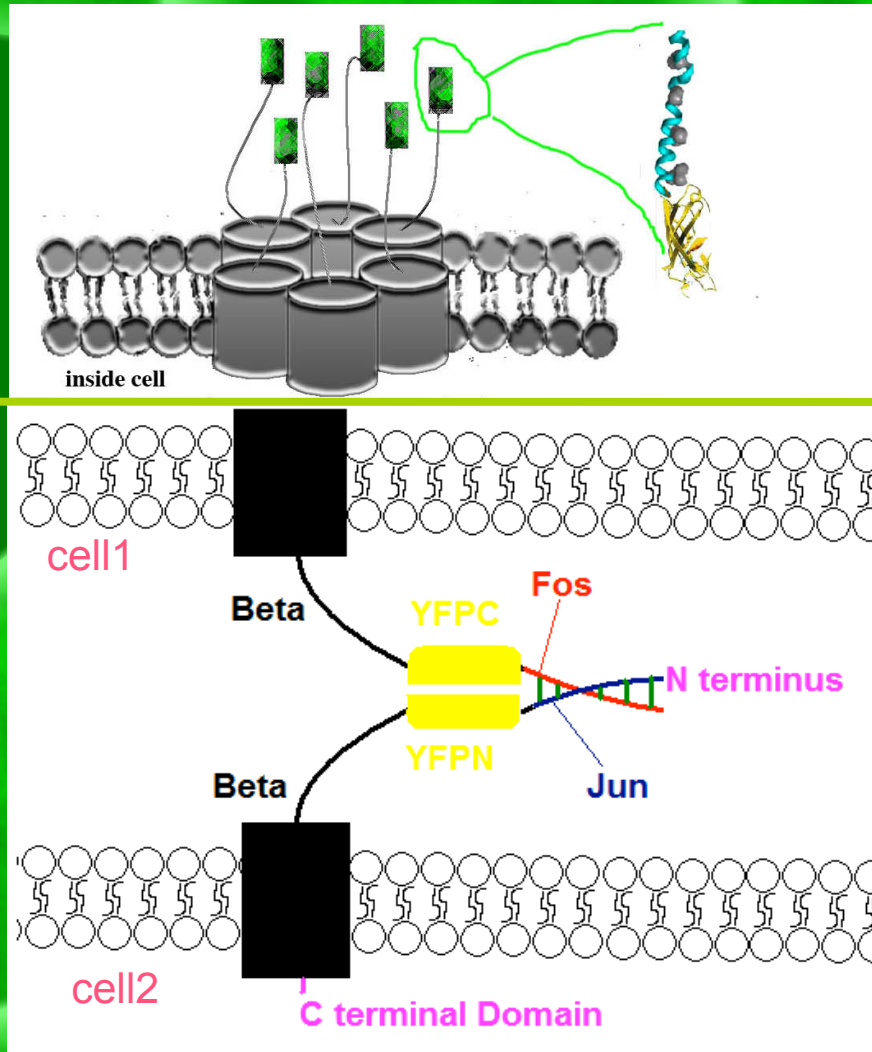
*Presented by: Julia Ishak,
Alexandre David, Adam Katolik*

Concept: Fluorescence Complementation



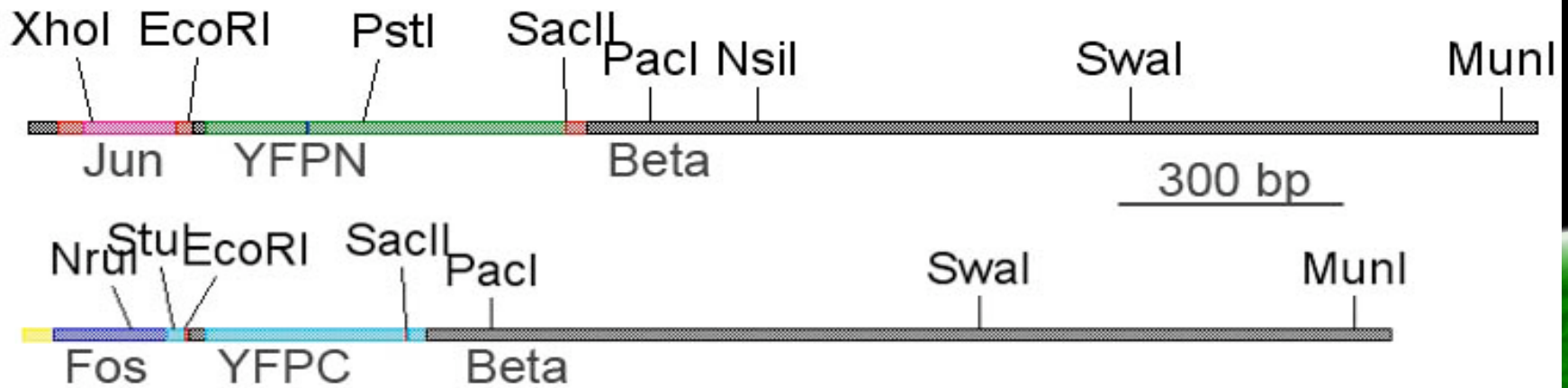
Modified from Hu and Kerppola, Nat Biotechnol. 2003
May;21(5):539-4

Can we use it OUTSIDE the cell?



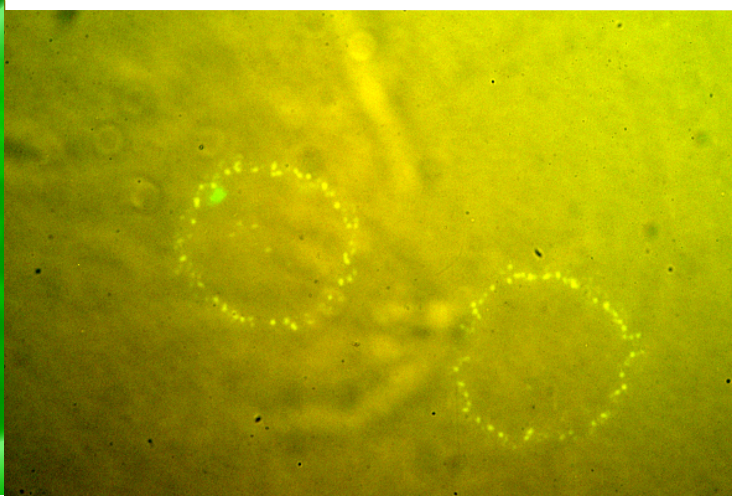
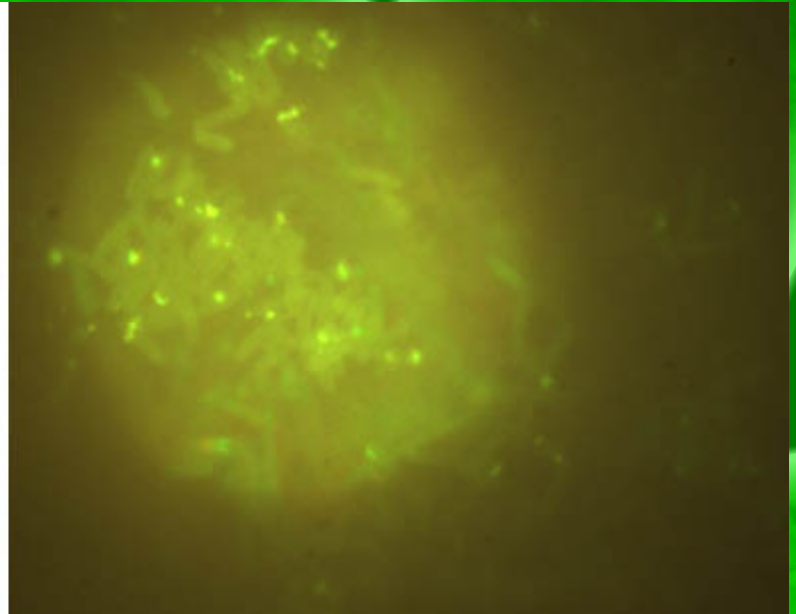
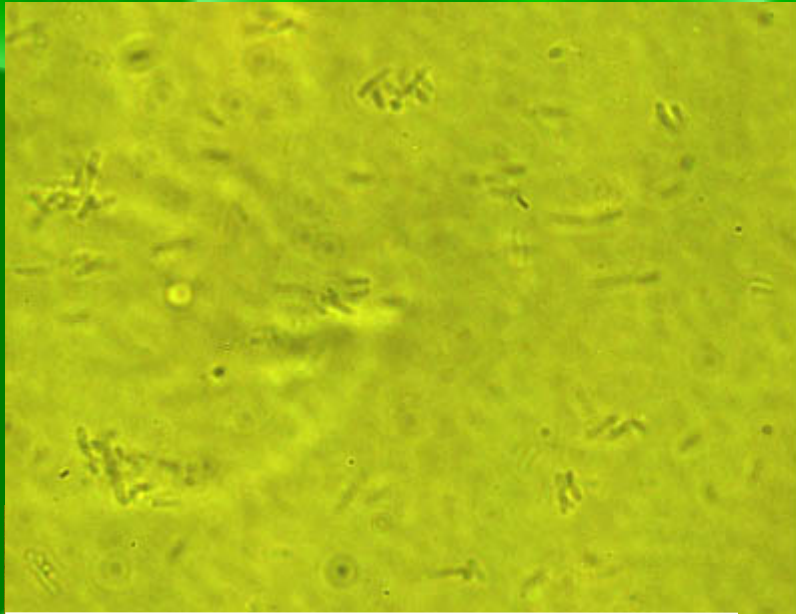
The beta-autotransporter domain of the immunoglobulin A (IgA) protease of *Neisseria gonorrhoea* (IgA beta) should transport the fragments to the surface of *E. coli* (Veiga, de Lorenzo, and Fernandez, J. Bacteriol. 2003 Sep;185 (18): 5585-90)

Constructs (not yet a brick!)



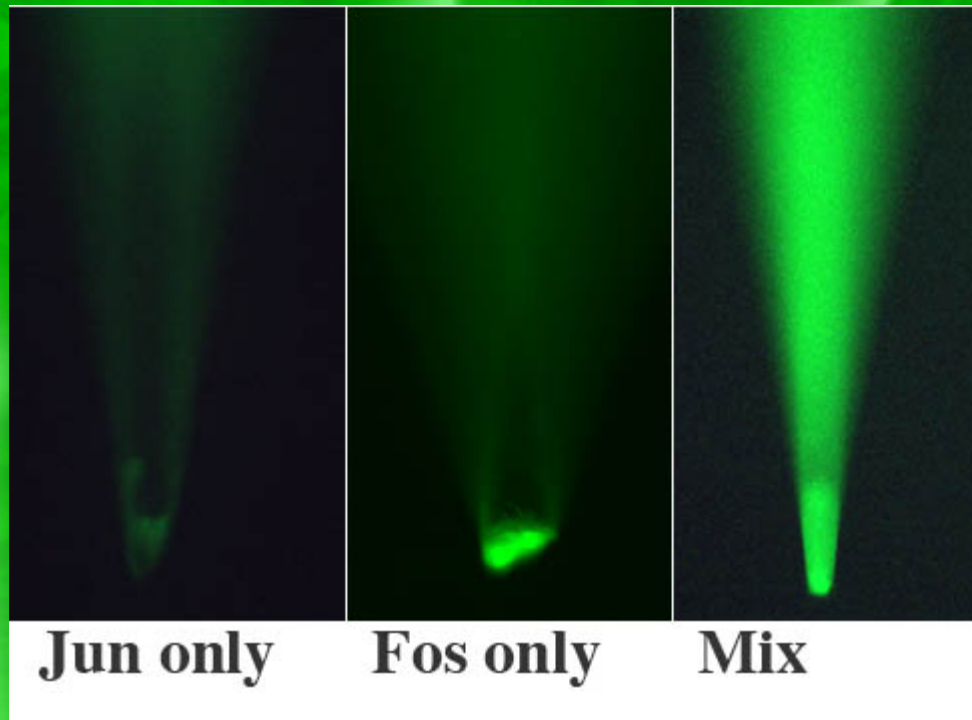
The two constructs are put into independent batches of cells and mixed before or during imaging

Premixed

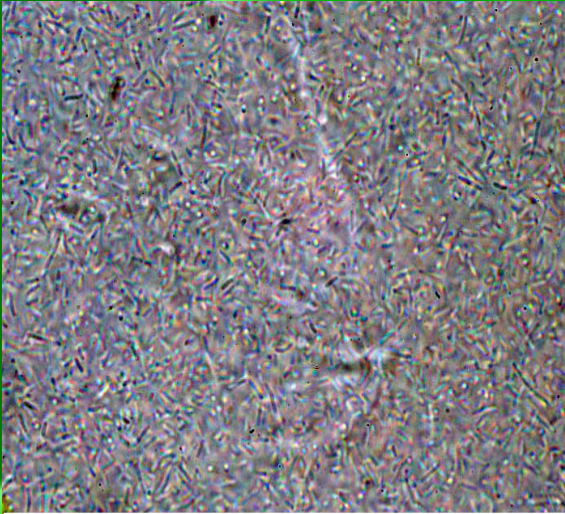


Strange geometric
shapes seen with flowing
cells

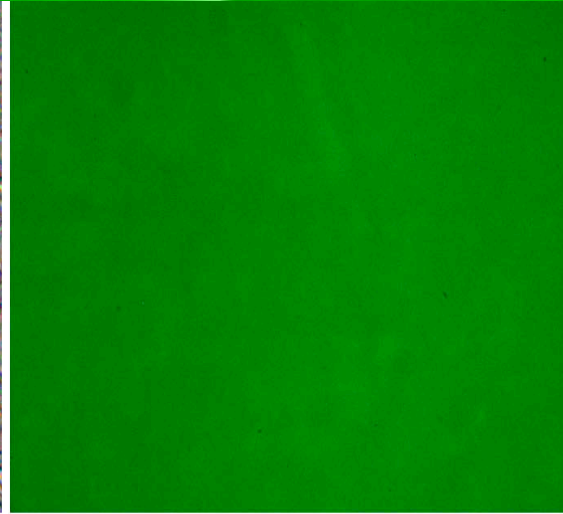
Mixed in a pipette tip



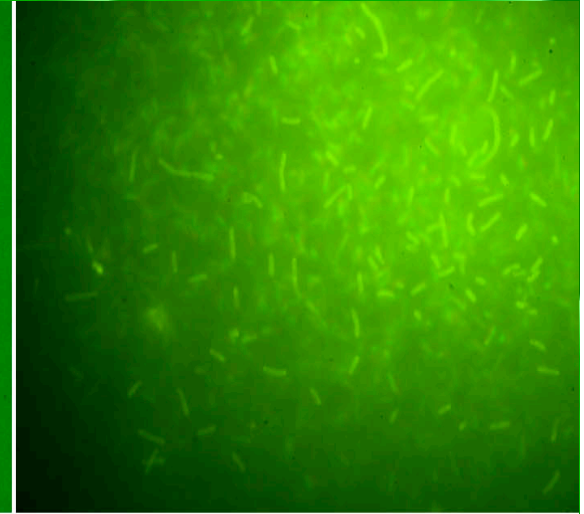
Squirting Fos into Jun



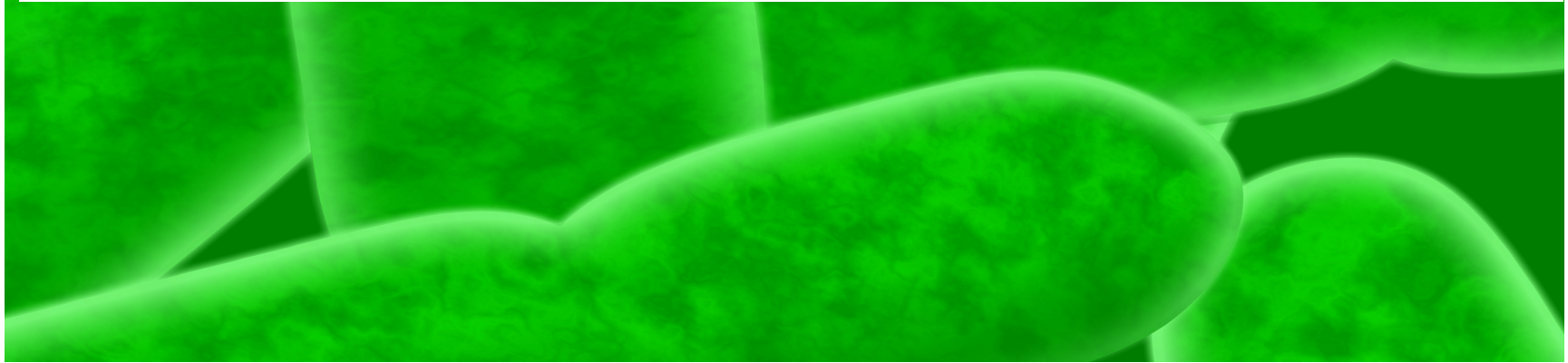
Phase contrast, Jun only



Fluorescence, Jun only



Fos added in upper right corner



Future work

- Make into BioBrick (must remove PstI sites!)
- Further explain fluorescence pattern and geometric shapes
- Determine circumstances under which fluorescence forms and is stable

Acknowledgements

- Team members: Team 1: Alexandre David, Ashwin Dixit, Julia Ishak, Adam Katolik, Belinda Kong
- Team 2: Ashwini Bapat, Brock Dumville, Jieun Kim, Aaron Lapierre, Jamie Schafer, Horia Vulpe, Josh Wright
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